

Assessing the Phenylketonuria Screening Program in Newborns, Iran 2015-2016

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Abstract- Phenylketonuria is one of the most important congenital disorders and an autosomal recessive metabolic disease that can cause irreversible brain damages, mental retardation, and cognitive disorders if left untreated. In order to reduce the genetic abnormalities caused by this metabolic disease, screening programs are implemented. The quality of the program must be properly assessed to achieve the objectives of this program if promoting children's health is of concern. The descriptive-analytic method is adopted here to assess the phenylketonuria screening program in practice in Chaharmahal and Bakhtiari province since 2012 and analyze the incidence and program coverage. The quality of the screening program is assessed through analyzing the time of diagnosis, beginning of the treatment and the healthcare centers' facilities with checklists. The parental and the staff awareness is assessed through knowledge measuring questionnaires. Cumulative incidence of phenylketonuria in Chaharmahal and Bakhtiari province from 2012 to 2015, is 1 in every 6662 live births. The program coverage across the region is 100%. The recorded on-time sampling index before 5 days of age, indicate 84.6 % in 2015 from 80% in 2012. The treatment begun before the newborn 4 weeks was over in all cases. Program sensitivity was 100 %, and its specificity was 99.9%. Staff awareness is fair with no impact on parental awareness. General quality of the screening program is appropriate, and as to sensitivity and on-time curing specificity, higher staff and parental awareness supervision are recommended as well.

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Introduction

Genetic and congenital abnormalities in the first month of birth are the main causes of body organs defects and death in newborns. Every year, around 7.6 million children are born to the world with genetic or congenital abnormalities 90% of whom belong to countries with a low per capita income (1).

The heavy burden caused by the neonatal diseases has made the states to plan and implement a screening program to diagnose neonatal and other types of metabolic diseases (2).

Phenylketonuria is a recessive autosomal disease caused by the deficiency of Phenyl Alanine Hydroxylases (PAH) enzyme and an increase in body Phenylalanine, and if not treated, leads to mental

disorders (3-4).

The outbreak ratio of this disease varies from 1:3000 to 1:60.000 across the nation (5-6).

According to the findings of the screening program conducted on 630.000 newborns under the supervision of 6 medical universities of 3 big provinces of Iran: Tehran, Fars, and Mazandaran during 1997-2010 the outbreak ratio of Phenylketonuria is 1:8000 (5).

As determined by the study carried out in the city of Isfahan, 2% (36 persons) of the 1611 mentally retarded patients who are kept in sanitariums suffer from Phenylketonuria (7).

In several other studies, this percentage is emphasized, and in some cases, it reaches 3% (8-9).

While the population of Iran is less than one-fourth of the population of USA, there exist 6 million disabled

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in both the countries. If the disease is not diagnosed and treated during the first year of birth, the child's IQ capacity may reduce by 50%, and it may cause problems in future treatments (10-11).

The marriages taking place within immediate family relatives increase the outbreak of the disease as well. The irreversible effects of this disease on the brain can be prevented through on-time diagnosis and a screening program followed by getting the treatment diet regime and controlling the Phenylalanine level in the blood (11-12-13); otherwise, families would face heavy financial problems.

The inability to afford the costly diet of the disease is a serious problem which many families of Phenylketonuria newborns have to face (12-13). Much of the state budget is planned to cover some of the expenses of the treatment, rehabilitation, maintenance and special diet of the patients despite the shortage of resources in the healthcare sector (10-13).

The screening of newborns at birth and the related healthcare sector the burdens on the state; therefore, assessing the efficiency and effectiveness of the screening program in the healthcare sector as a health factor is essential. In order to enhance the quality of services and to economize, the organizations have to include the assessment process in their schedules and conduct the implementation in a scientific and technological manner (14-15).

By implementing the screening program on newborns throughout the nation, with the objective of accomplishing almost 100% coverage and completing the studies on other types of metabolic diseases together with adopting appropriate plans by determining the outbreak and related costs' level, it may be possible to incorporate the screening program of other diseases in the healthcare sector.

In order to achieve the objectives of this program and promote health in families, the accountability and high quality of the screening program must be proved. In this study, attempt is made to assess the different parts of the program in Chaharmahal and Bakhtiari province, and apply the findings in determining the strong and weak points of the program and adopting policies to remove the weak point and promote the strong point.

Materials and Methods

Participants

In 2015, 200 workers from different healthcare centers participated in a descriptive-analytical study subject to

conditions of at least 6 months of work experience in healthcare centers and willingness to participate. To begin with, with respect to every city's population the quota of each city is determined on a random sampling basis. The participants consist of 10 workers from Koohrang, 16 from Kiar, 15 from Ardal, 40 from Lordegan, 24 from Farsan, 25 from Boroojen and 70 from Shahrekord, Saman and Ben. The participants are required to answer the knowledge assessment questionnaire composed of 20 questions, and each correct answer equals to one point, that is, a ranking of 0-20. The validity of the questionnaire is confirmed by the experts in a scientific manner. In order to assess the reliability of the questionnaire a pilot study is conducted and the correlation coefficient 0.76 is obtained.

The count of the diagnosed and undiagnosed patients is determined based on the information available in the healthcare center of the province. Since the beginning of this program, the positive cases of the reference laboratory which proved a negative result in the next examinations are annulled. The parents are required to answer the knowledge and care regarding the newborns with Phenylketonuria in the questionnaire in accordance with the census. This questionnaire is composed of 16 questions, and each correct answer is worth 1.25 points, thus a ranking from 0-20. The validity of the questionnaire is confirmed by the experts based in a scientific manner. In order to assess the reliability of the questionnaire the split-half method is applied and the correlation coefficient 0.68 is obtained.

In the program reference laboratories, the 200 Guthrie card samples collected from the healthcare centers are examined. The quality of sample collecting process is assessed through a reference to the thyroid screening program checklist (16).

The availability of the materials necessary for sample collecting process consisting of Guthrie card, Lancet, alcohol pad and the forms used in healthcare centers at least for the past one month are assessed.

The data is assessed by SPSS software, the Relative Frequency is applied for the description and for the analysis of the Independent t-test, and Pearson Correlation Coefficient are applied.

Results

In this study, the findings obtained from the implementation of Phenylketonuria screening program beginning June 2012 and ending June 2015 throughout the nation are analyzed in Chaharmahal and Bakhtiari province and tabulated in Table 1.

Table 1. The distribution of Phenylketonuria in the four consecutive years

Year	Phenylketonuria cases	Live Births
2012	1	13022
2013	4	19612
2014	3	19753
2015	3	20893

The cumulative outbreak ratio of the disease in the four consecutive years is 1:6662 live births. From the 20 cases of Phenylketonuria, 9 cases were diagnosed before the implementation of the program, 11 cases during the program and 2 cases were born dead; therefore, the frequency of Phenylketonuria consists of 18 patients.

Since the beginning of the program, during the four consecutive years, heel blood samples are collected from each newborn, and the coverage of the program is at 100%; however, the on-time sample collecting index (sample collecting in the first 5 days of birth) shows a different result in the four consecutive years.

Table 2. On time sample collecting index

Year	On time sample collecting percentage	Delayed sample collecting percentage
2012	80%	20%
2013	81%	19%
2014	83%	17%
2015	84.6%	15.4%

The treatment for each of the Phenylketonuria newborns during the four consecutive years began

before they reached four weeks of age with differences in beginning time of the treatment.

Table 3. Treatment beginning time

Year	Less than 2 weeks	2-3 weeks
2012	(1)100%	0
2013	(2)50%	(2)50%
2014	(1)33.3%	(2)66.7%
2015	(2)66.7%	(1)33.3%

No cases of Transient hyperphenylalaninemia are observed during the implementation of the program. Seven false positive cases of ELISA are revealed: one in 2012, one in 2013, 2 in 2014 and 3 in 2015 which are then rejected after being identified as negative through HPLC test. Therefore, the sensitivity of the program during the four consecutive years is 100% with 99.9% specificity. The negative predictive value in the four consecutive years is 100%, but the positive predictive value varies in each consecutive year by 50% in 2012, 80% in 2013, 60% in 2014 and 50% in 2015. Throughout the program, the negative false result is registered at 0% and the positive false result at less than 0.01%.

The obtained positive and negative predictive values of the HPLC test is 100%, that is, all the positive cases have the disease, and all the negative cases don't have the disease.

According to the assessments conducted by the

provincial Health Department, the instructional texts and resources were available in all the healthcare centers to train the personnel.

The average age of the 200 participants is 39.12 ± 7.79 . The average working experience of the workers is 16.69 years with 8.88 standard deviations (SD), and the mean score of knowledge from 0 to 20 scores is 10.88 with 4.92 (SD). From the 200 participants of the study, 117 are female (58%), and 83 are male (41.5%) workers.

There exists no statistical significance between the mean score of knowledge and the average age of the participants; $P=0.518$.

There exists no statistical significance between the mean score of knowledge and the working experience of the participants; $P=0.336$.

There exists no statistical significance between the mean score of knowledge and the gender of the participants; $P=0.352$.

Table 4. The mean score of knowledge

Gender	Knowledge mean	SD	P
Female	11.1	4.8	0.352
Male	10.14	5	

The parents of the newborn with Phenylketonuria are provided with information on the disease by the hospital and healthcare center personnel. In one case the parents gathered the necessary information from the internet. The mean score of knowledge of parents within 0 to 20 is 15.7 with 1.1 (SD).

In the 20 cases of this study, two cases of the patients are first relatives of the first degree (10%), 9 cases are relatives of the second degree or higher (45%), and the

other 9 cases are not relatives (45%).

As to the average time regarding sample delivery to the laboratory not all were sent immediately but within 48 hrs.

The positive cases are reported by phone to the sample collecting centers, and the negative cases are reported in written form to the centers within 2 weeks.

The sample collecting process in the healthcare centers is checked in terms of quality, Table 5.

Table 5. Checking the sample collecting process quality

Question	Yes	No
Were 4 circles of Guthrie papers completed with blood sample	124(62%)	76(38%)
Were the samples in circular shape?	86(43%)	114(57%)
Were the blood spots diameter more than 5 mm?	14(7%)	186(93%)
Whether there were more than one blood stains in each circle?	185(92.5%)	15(7.5%)
Were the blood spots without the fingerprint?	200(100%)	0
Did the blood spots look identical on both sides of the cards?	189(94.5%)	11(5.5%)
Were the blood spots overlap?	188(94%)	12(6%)

Discussion

The Phenylketonuria screening program for newborns in Chaharmahal and Bakhtiari province is a process going on since 2012. Since the begging of the program until 2015, 73280 newborns have been exposed to the screening program, and the 11 diagnosed cases of PKU are under treatment.

Here, it is revealed that the screening program coverage in the province is 100%. The screening program coverage of Brazil varies in different years: 72% in 2003, 78.28 in 2005 and 78.93 in 2007 (17-18-19). In Parana, the coverage increased from 86.99% to 99.01% in 1996-2001 (20). In a study conducted in France, it is revealed that the screening program coverage increased in a progressive manner and in 1980 reached its maximum percentage (21). The outstanding coverage percentage in Chaharmahal and Bakhtiari province is the outcome of the excellent work performed in the healthcare centers of the nation.

Based on the findings of this study, the outbreak ratio of Phenylketonuria in Chaharmahal and Bakhtiari province is 1:6662 in live births. This ratio varies in different countries: the lowest outbreak ratio is found in Finland (1:200000) and Japan (1:125000) which is due

to the genetics and the highest outbreak ratio is found in Arabian countries (more than 1:5000) and Turkey (1:2600) where the marriages take place within immediate family relatives (22). The outbreak ration of the disease is 1:8690 in Brazil and 1:10000 in Europe (23-24-25). Among the European countries, the outbreak ratio of the disease is surprisingly high in Ireland and Western Scotland (1:4500) making it one the highest ratios (26). In China, this ratio is 1:18000 (27).

In a study conducted in Fars province in 2010, it is revealed that the outbreak ratio of PKU in Shiraz and Eghlid is 1:4698 and 1:384, respectively, which is due to the existence of a pathogen genetic factor (28).

In 8633 births in various hospitals of Tehran, the outbreak ratio is estimated as 1.1:10000. In 1544 newborns with the symptoms of the metabolic disease, the outbreak ratio is 1:3672 (29). In the city of Isfahan, the outbreak ratio among the 1611 mentally retarded patients is 20% (36:1611), (30).

Through the comparison of the findings of various studies, it can be deduced that the outbreak ratio in Chaharmahal and Bakhtiari province is close to the national average. The outbreak ratio in this province is high due to the 100% coverage of the screening program and the sensibility and appropriateness of

HPLC testing which diagnose all the newborns with the disease.

According to this study, the on-time sample collecting index (first 3-5 days of birth) is enhanced from 80% to 84.6% in 2012-2015. Iran is in a better position when compared to other nations, with respect to the sample collecting timing.

In a study conducted in Brazil in 2014 it is reported that the filter paper collecting process took 2-30 days and in 26.74% of the cases, the screening program is conducted on 2-6 years old children (23). The average age of the newborns who were exposed to the screening program in Mexico is 18 days (31).

According to this study, the treatment for the PKU newborns diagnosed in the screening program began within 4 weeks: 6 newborns less than 2 weeks (54.5%) and 5 newborns in their 2-3rd weeks (45.5%). In the study conducted in Brazil (2004), in 71.9% cases, the treatment began in 30 days and in 15.6% after 60 days (32). The appropriate timing in collecting samples and beginning the treatment in the province is the outcome of the consistency and the excellent management.

The sensibility and significance of HPLC is 100% in this province. The positive predictive value of ELISA testing varies in four consecutive study years, which is adjusted through HPLC. The total significance of the program is 99%.

In the study carried out in France, from the 21.5 million newborns exposed to the PKU screening program with a 99.3% sensibility. Two newborns were not exposed to the Guthrie test, 2 other patients disappeared before the testing, and in case of 6 newborns a false negative result is detected (33). Several other studies reported a similar ratio of false negative results with diverse sensitivities ranging from 98% to 99.2% (34-35-36). According to various studies, the ratio of false negative results is reduced over time (37). The high sensitivity and significance of the program in this study is the outcome of the appropriate management system and the reliable reference laboratories with experienced personnel.

In this study, more than 50% of the newborns of the diagnosed cases are the offspring of the marriages taking place within immediate family relatives. In the studies conducted in Mashhad and Mazandaran provinces, 80% (high) and 60% (moderate) of the parents are relatives, respectively. In some other studies, 82% of the parents are not relatives (38-39-40).

Considering the existence of the disease in other members of the family, two newborns suffering from the disease are sisters in this study, which corresponds to the

studies of 12% in Mashhad, 10% in Shiraz, 12% in Mexico (28-29-30-31).

The mean score of knowledge about Phenylketonuria screening program of the healthcare center workers is average, which is corresponds to the study of hypothyroid screening program in newborns. However, this score is a warning in order to revise the worker training programs (16).

From the findings of this study, it is deduced that the healthcare center workers did not play an important role in enhancing the knowledge of the parents about the disease and the treatment procedure. This may be due to the fact that the workers are not trained enough and refuse to give instructions to the parents of the newborns and do not collaborate with the healthcare centers after the diagnosis of the disease and enrolled at the hospital care.

Although the knowledge score of the workers is not high, all of the newborns are diagnosed and cured which is the outcome of conducting the screening in an appropriate manner and the excellent collaboration of the laboratory and hospital personnel.

The quality of the sample collecting procedure is far from ideal, but based on the high sensitivity and significance of the program the least requirements to collect the samples are observed. Although the required standard for filling out the four circles is not observed, the 5mm and 3mm samples provided the least necessary volume.

Despite the existing problems, the materials necessary to collect the samples in the healthcare centers are provided, and there exists a direct cooperation among healthcare centers' personnel.

Attempt is made here to determine the causative weaknesses of the Phenylketonuria screening program in an objective manner.

In accordance with the findings of this study, the quality of the Phenylketonuria screening program in the province is met in terms of collecting the samples, on- time diagnosis and immediate treatment operations. The knowledge score of the healthcare center workers is insufficient, which affects the efficiency of the program. Although the knowledge score of the parents is higher than the average, care must be taken in order to improve the knowledge score of the parents and the workers. The parents are required to collaborate with the healthcare centers, and the mothers must get the required instructions regarding the program before giving birth; therefore, the program manager is required to have more control over the health care center workers.

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References

1. Kaur A, Singh JR. Chromosomal abnormalities: Genetic disease burden in India. *Int J Hum Genet* 2010;10:1-14
2. Loeber JG. Neonatal screening in Europe; the situation in 2004. *J Inherit Metab Dis* 2007;30:430-8.
3. Williams RA, Mamotte CDS, Burnett JR. Phenylketonuria: an inborn error of phenylalanine metabolism. *Clin Biochem Rev* 2008;29:31-41.
4. Birk Møller L, Nygren AO, Scott P, Hougaard P, Bieber Nielsen J, Hartmann C, et al. Low proportion of whole exon deletions causing phenylketonuria in Denmark and Germany. *Hum Mutat* 2007;28:207.
5. Hatam N, Askarian M, Shirvani S, Pourmohammadi K. Cost Utility of Neonatal Screening Program for Phenylketonuria in Shiraz University of Medical Sciences. *Hakim Res J* 2014;16:329- 36.
6. Koochmeshgi J, Bagheri A, Hosseini Mazinani SM. Incidence of phenylketonuria in Iran estimated from consanguineous marriages. *J Inherit Metab Dis* 2002;25:80-1.
7. Vallian S, Barahimi E, Moeini H. Phenylketonuria in Iranian population: a study in institutions for mentally retarded in Isfahan. *Mutat Res* 2003;526:45-52.
8. Holmgren G, Larsson A, Palmstierna H, Alm J. The frequency of PKU and hyperphenylalaninemia in Sweden—a study in institutions for the mentally retarded as well as in neonates. *Clin Genet* 1976;10:313-8.
9. DiLella AG, Woo SL. Molecular basis of phenylketonuria and its clinical applications. *Mol Biol Med* 1987;4:183-92.
10. Watson MS, Mann MY, Lloyd-Puryear M, Rinaldo P, Howell RR. American College of Medical Genetics Newborn Screening Expert Group. Newborn screening: toward a uniform screening panel and system—executive summary. *Pediatrics* 2006;117:5296-307
11. Burton BK, Leviton L, Vespa H, Coon H, Longo N, Lundy BD, et al. A diversified approach for PKU treatment: routine screening yields high incidence of psychiatric distress in phenylketonuria clinics. *Mol Genet Metab* 2013;108:8-12.
12. Gentile JK, Ten Hoedt AE, Bosch AM. Psychosocial aspects of PKU: hidden disabilities—a review. *Mol Genet Metab* 2010;99:64-7.
13. Camp KM, Lloyd-Puryear MA, Yao L, Graft SC, Parisi MA, Mulberg A, et al. Expanding research to provide an evidence base for nutritional interventions for the management of inborn errors of metabolism. *Mol Genet Metab* 2013;109:319-28.
14. Lugner LK, Mollema L, Ruijs WLM, Hahne SJM. A cost utility analysis of antenatal screening to prevent congenital rubella syndrome. *Epidemiol Infect* 2010;138:1184.
15. Dhondt JL, Farriaux JP, Saille JC, Lebrun T. Economic evaluation of cost-benefit ratio of neonatal screening procedure for phenylketonuria and hypothyroidism. *J Inherit Metab Dis* 1991;14:633-9.
16. Kasiri KA, Ganji F, Beigi R, Hashemi E. Evaluation of congenital hypothyroidism screening program in urban and rural health centers in Chaharmahal and Bakhtiari. *J Shahrekord Univ Med Sci* 2014;16:89-94.
17. Ramalho RJR, Valido DP, Aguiar-Oliveira MH. Avaliação do Programa de Triagem para o Hipotireoidismo Congênito no Estado de Sergipe. *Arq Bras Endocrinol Metab* 2000;44:157-61.
18. Ramalho RJ, Ramalho AR, Oliveira CR, Aguiar-Oliveira MH. Evolution of the screening program for congenital hypothyroidism and phenylketonuria in Sergipe State from 1995 to 2003. *Arq Bras Endocrinol* 2004;48:890-6.
19. Ramalho AR, Ramalho RJ, Oliveira CR, Santos EG, Oliveira MC, Aguiar-Oliveira MH. Neonatal screening program for congenital hypothyroidism in northeast of Brazil: criteria, diagnosis and results. *Arq Bras Endocrinol* 2008;52:617-27.
20. Souza CFM, Schwartz IV, Giugliani R. Neonatal screening of metabolic disorders. *Ciênc Saúde Coletiva* 2002;7:129-37.
21. Abadie V, Berthelot J, Feillet F, Maurin N, Mercier A, de Baulny HO, et al. Neonatal screening and long-term follow-up of phenylketonuria: the French database. *Early Hum Dev* 2001;65:149-58.
22. Williams RA, Mamotte CDS, Burnett JR. Phenylketonuria: an inborn error of phenylalanine metabolism. *Clin Biochem Rev* 2008;29:31-41.
23. Ramalho AR, Ramalho RJ, Oliveira CR, Magalhães MM, Santos EG, Sarmento PM, et al. Evaluation of effectiveness and outcome of PKU screening and management in the State of Sergipe, Brazil. *Arq Bras Endocrinol* 2014;58:62-7.
24. Loeber JG. Neonatal screening in Europe; the situation in 2004. *J Inherit Metab Dis* 2007;30:430-8.
25. Woolf LI. The high frequency of phenylketonuria in Ireland and western Scotland. *J Inher Metab Dis* 1978;1:101-3.
26. Liu Sr, Zuo Qh. Newborn screening for phenylketonuria in eleven districts. *Chin med J (Engl)* 1986;99:113-8.

27. Kaye CI, Committee on Genetics, Accurso F, La Franchi S, Lane PA, Hope N, et al. Newborn screening fact sheets. *Pediatrics* 2006;118:934-63.
28. Karamifar H, Ordoei M, Karamizadeh Z, Amirhakimi GH. Incidence of neonatal hyperphenylalaninemia in Fars Province, Southern Iran. *Iran J Pediatr* 2010;20:216-20.
29. Farhud DD, Kabiri M. Incidence of phenylketonuria in Iran. *Indian J Pediatr* 1982;49:685-8.
30. Golbahar J, Karamizadeh Z, Honardar Z. Selective screening of amino acid disorders in the south-west of Iran, Shiraz. *J Inher Metab Dis* 2002;25:519-21.
31. Vela-Amieva M1, Ibarra-González I, Fernández-Lainez C, Monroy-Santoyo S, Guillén-López S, Belmont-Martínez L, et al. Cause of delay in referral of patients with phenylketonuria to a specialized reference center in Mexico. *J Med Screen* 2011;18:115-20.
32. Brandalize SD, Czeresnia D. Evaluation of the program for prevention and health promotion in phenylketonuria patients in Brazil. *Revista de Saúde Pública* 2004;38:300-6.
33. Abadie V, Berthelot J, Feillet F, Maurin N, Mercier A, de Baulny HO, et al. Neonatal screening and long-term follow-up of phenylketonuria: the French database. *Early Hum Dev* 2001;65:149-58.
34. Holtzman C, Slazyk WE, Cordero JF, Hannon WH. Descriptive epidemiology of missed cases of phenylketonuria and congenital hypothyroidism. *Pediatrics* 1986;78:553-8.
35. Hanley WB, Demshar H, Preston MA, Borczyk A, Schoonheydt WE, Clarke JT, et al. Newborn phenylketonuria (PKU) Guthrie (BIA) screening and early hospital discharge. *Early Hum Dev* 1997;47:87-96.
36. Verkerk PH. 20-year national screening for phenylketonuria in The Netherlands. National Guidance Commission PKU. *Nederl Tijdschr Geneesk* 1995;139:2302-5.
37. Smith I, Cook B, Beasley M. Review of neonatal screening programme for phenylketonuria. *BMJ* 1991;303:333-5.
38. Senemar S, Ganjekarimi H, Fathzadeh M, Tarami B, Barzgar M. Epidemiological and clinical study of phenylketonouria (PKU) disease in the national screening program of neonates, Fars Province, Southern Iran. *Iranian J Publ Health* 2009;38:58-64.
39. Habib A, Fallahzadeh MH, Kazeroni HR, Ganjkarimi AH. Incidence of Phenylketonuria in Southern Iran. *Iran J Med Sci* 2010;35:137-9.
40. Eshraghi P, Abaskhanian A, Mohammadhasani A. Characteristics of patients with phenylketonouria in Mazandaran Province, Northern, Iran. *Casp J Int Med* 2010;1:72-4.